

PLOIDY OF SOME NON-SPHERICAL NUCLEI IN COCKROACHES CALCULATED FROM PHOTOMETRICALLY MEASURED DNA CONTENT¹

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ABSTRACT

A modified photometric technique for measuring the DNA content in nuclei was devised to estimate the degree of ploidy in the large flattened (oblate spheroidal) nuclei of the male accessory gland in the American cockroach, *Periplaneta americana* (L.), and the German cockroach, *Blattella germanica* (L.). This technique involves measuring diameters of the nuclei as well as light transmission through the nuclei in two dimensions rather than one as with spherical nuclei. The measurements are then combined to form an "average nucleus." Regular photometric methods were employed on the haploid spermatid used as the standard for comparison. The modified technique produced DNA values that were nearly perfect multiples of the standard. The calculations revealed 16-ploid and 32-ploid nuclei in *P. americana* and 8-ploid and 16-ploid nuclei in *B. germanica*.

Examinations of stained tissue sections and smears of the American cockroach, *Periplaneta americana* (L.) and the German cockroach, *Blattella germanica* (L.), revealed especially large nuclei in certain tissues. The largest nuclei observed in either species were located in cells of the accessory glands of the male reproductive system. Because of their large size and unusual shape, I wondered whether these nuclei contained the normal diploid complement of chromosomes or whether they were polyploid, and if the latter, to what extent.

All the accessory gland nuclei observed were non-dividing. All were without heteropycnotic bodies, distinct chromosomes, or any other visible means of determining the degree of ploidy in them. Nuclear volume could have been calculated, but this method of determining ploidy in insect tissues is not always accurate (Schrader and Leuchtenberger, 1950).

One method of estimating ploidy in non-dividing nuclei was developed by Pollister and Ris (1947), and since then has been used successfully on insects and other organisms by numerous researchers (Merriam and Ris, 1954; Stich, 1962; Swift, 1950b; Vogl, 1955). The apparatus employed measures the relative amount of deoxyribonucleic acid (DNA) in individual nuclei with a sensitive light meter. As DNA is a constant component of the chromosomes, comparative measurements between nuclei of known and unknown ploidy can be made. The technique employs the use of the Feulgen staining method, a method that is specific for DNA, if used correctly (Swift, 1950b).

As the equipment used by Vogl (1955) was available to me, I prepared tissues and developed a modified photometric technique in order to estimate the degree of ploidy in the male accessory gland nuclei of *P. americana* and *B. germanica*.

TISSUE PREPARATION

The accessory gland of the male genital system in cockroaches consists of a mass of short and long, sack-like utriculi, and is located at the anterior end of the ejaculatory duct. Its general morphology is discussed by Snodgrass (1937).

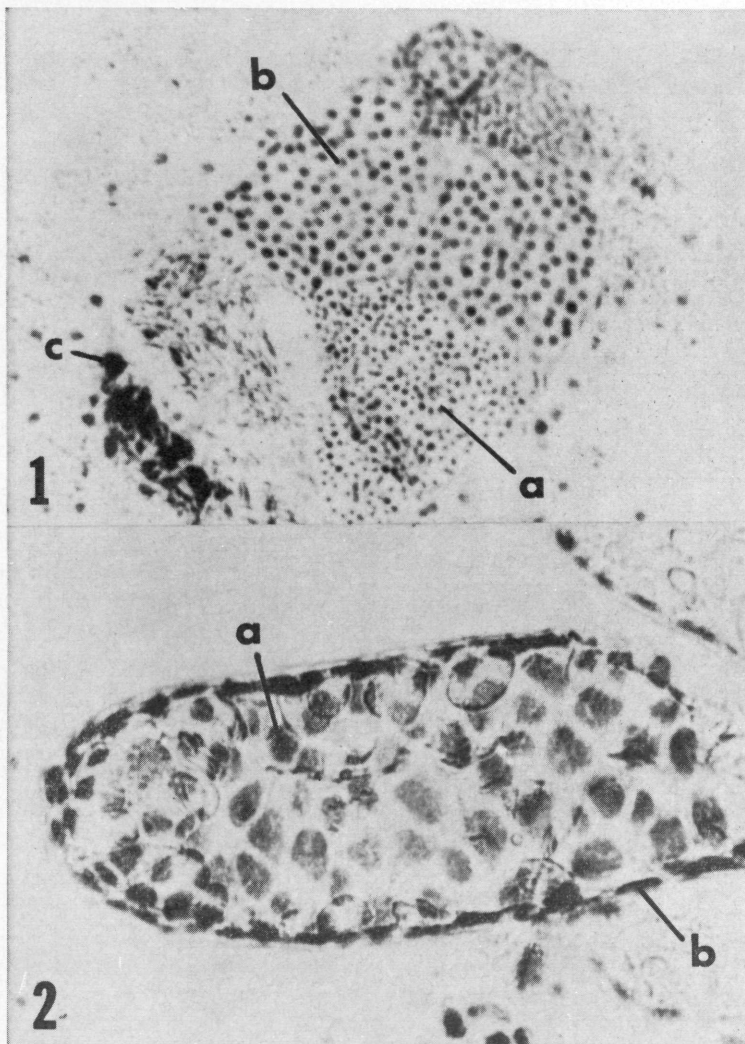
Photometric measurements on nuclei of unknown ploidy are valueless unless compared to measurements from some standard of known ploidy in the same

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²Located at the Station's East Lansing, Mich., field unit which is maintained in cooperation with Michigan State University. This study was made while a graduate student at Marquette University, Milwaukee, Wisc.

organism. The spermatid in the testis, which is haploid, was selected for the standard.

Five adult or last instar male cockroaches of each species were selected as test specimens. The testes and accessory glands were dissected out of the abdomens of the American cockroaches. Because the German cockroaches are much smaller, their abdomens were severed from the rest of their bodies and treated as an aggregate. All tissues were fixed in 20 per cent formalin for 38 hrs at room temperature. Formalin was used to preserve the nuclei in a homogeneous state to reduce the error in the photometer readings due to the normally uneven distribution of chromatin (Swift, 1950b; Vogl, 1955).



FIGURES 1 and 2. Sections of testes and accessory glands of *Periplaneta americana*. 1. lobe of testis showing: a., spermatids, b., secondary spermatocytes, c., spermatozoa. 2. Utriculus of male accessory gland showing large flattened nuclei: a., viewed from the broadside, b., viewed from the edge. Photometric measurements were made on tissues cut in longitudinal and cross sections.

Following fixation, the tissues were washed gently in cool running water for several hours to remove the unbound formalin. During the next two-day period, the tissues were dehydrated in a graded series of ethanol and embedded in tissuemat. The testes were sectioned at $6\ \mu$ and the utricular glands at $20\ \mu$ for *P. americana*. *B. germanica* tissues were sectioned at $1.6\ \mu$. After affixing the sections to slides, the tissues were hydrolized in normal HCl at $60\ ^\circ\text{C}$ for 8 min for *Blattella* and 10 min for *Periplaneta*, then stained with leuco-basic fuchsin (Feulgen) for 30 min, and mounted permanently.

Figures 1 and 2 show photomicrographs of stained testis and accessory gland sections from *P. americana*. Photometric measurements were made on tissues cut in longitudinal and cross sections.

PHOTOMETRIC MEASUREMENTS ON THE TESTES

The equipment used is described in detail by Vogl (1955). The core method of measuring the relative amount of DNA is explained in detail by Swift (1950b). It is described here briefly in order to make clearer certain necessary modifications in the measurement of the accessory gland nuclei. Although most measurements were made on the spermatids, a few were also made on the secondary spermatocytes for comparison. Only whole spherical nuclei were measured. Two diameter measurements at right angles to each other were measured with an ocular micrometer ($1\ \text{unit} = 0.125\ \mu$), and averaged if they differed (table 1). The photometer reading was taken through a central 20-unit-wide core of the nucleus, followed by a reading nearby through a 20-unit-wide blank, unnucleated part of the cytoplasm. The cytoplasm remained nearly colorless after Feulgen staining. Figure 3A, B, illustrates the measurements on these nuclei.

All photometer measurements were taken through a $0.620\text{-}\mu$ interference filter with a half-band width of $30\ \mu$ placed across a light source set up for Köhler illumination. An interference filter of $0.620\ \mu$ was used to reduce the error normally made by measuring densely straining nuclei at the $0.540\ \mu$ peak of the Feulgen absorption curve (Swift, 1950a; Patau, 1952). Light transmission (T) was calculated from $T = I_n/I_b$ where (I_n) is the light intensity reading of the nucleus and (I_b) is the light intensity reading of the blank. Since (T) does not vary directly with the amount of absorbing substance, the extinction (E), which does very directly, was calculated for each nucleus from $E = 1/\log T$. The extinction (E) is then divided by that fraction (F) of the total nuclear volume represented by the core volume, in order to get the extinction value for the total nuclear volume (see Swift, 1950b, for calculating F). This value (E/F) represents the amount of DNA in arbitrary units in each whole nucleus.

PHOTOMETRIC MEASUREMENTS ON THE ACCESSORY GLANDS

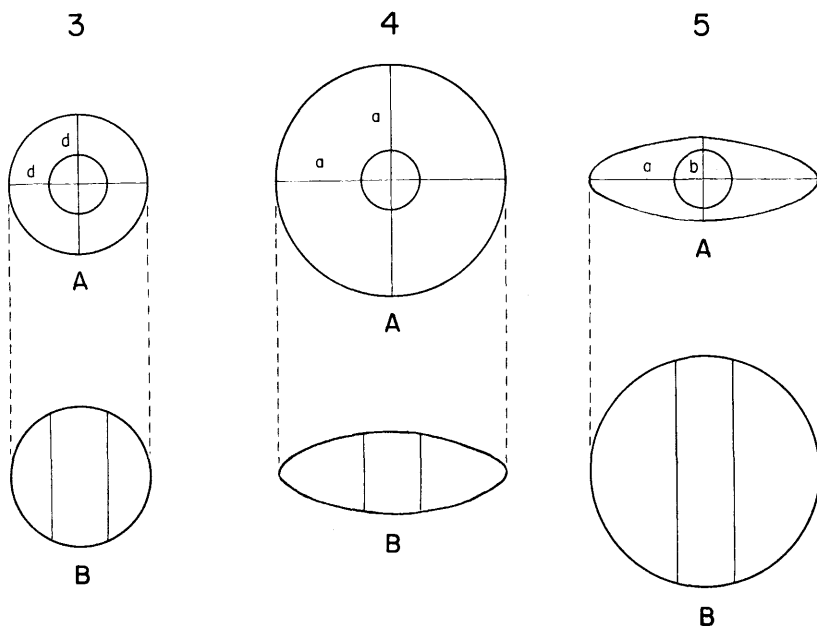
Whole nuclei in the male accessory glands appeared nearly spherical in certain tissue sections; they were nearly circular in outline as viewed through the microscope. In other sections (Fig. 2), they appeared elongate or fusiform. Actually, the nuclei were highly flattened spheres or oblate spheroids. Because of this shape, the following modified technique was developed in order to determine the relative amount of DNA.

Only the most symmetrical, whole nuclei, were chosen for measurements. First, two diameters at right angles were measured from the broadside of several nuclei (fig. 4A). A photometer reading from each was taken through a central 20-unit core (fig. 4A and B), followed by a blank reading in an unnucleated region nearby. Then, several nuclei were measured on edge. The long diameter and short diameter of each were measured at right angles to each other (fig. 5A). As before, a central 20-unit core photometer reading was taken, followed by a blank reading (fig. 5A and B). Light transmission (T_1) and (T_2) and extinction (E_1)

and (E_2) for broadside and edge readings respectively were calculated in the manner used on testis' nuclei.

Long diameter measurements were charted as histograms and found to occupy two frequency classes. This held true for both species of cockroaches (table 1). The measurements were separated into their respective classes for further computations.

As diameters of nuclei in tissue sections can be measured in only one plane, the volume of these oblate spheroidal nuclei in the broadside plane can not be calculated by normal means. Diameters from all the nuclei in each class were combined to form an "average nucleus." The "average nucleus" then had a



FIGURES 3-5. Diagrammatic illustration of measurements on the sample nuclei. 3. spermatid nucleus; 4. accessory gland nucleus measured from the broadside; 5. accessory gland nucleus measured from the edge. A. nuclei as viewed and measured through the microscope; B. same nuclei viewed at right angle. Letters: a =long diameter; b =short diameter; d =diameter. Central circles and parallel lines indicate the 20-unit-wide core through which transmission was measured.

mean long diameter (from measurements on nuclei in the broadside and edge planes) and a mean short diameter (from measurements on nuclei in the edge plane only). With this, the volume (V) of the "average nucleus" (i.e., an oblate spheroid) can be calculated from

$$V = \frac{4}{3}\pi a^2 b$$

where a is the mean long diameter and b is the mean short diameter. Further, an oblate spheroid can be converted to a sphere of equal volume by

$$r = \sqrt{\frac{a^2 b}{8}}$$

where r is the radius of the sphere.

Then, considering the "average nucleus" as if it was a sphere, the fraction $[(F)]$ of the total nuclear volume represented by the core volume (F) was calculated like that for the spherical nuclei of the testis. A mean extinction (E_m) was obtained from the arithmetic average of the measurements taken from nuclei in both planes ($E_m = \frac{E_1 + E_2}{2}$). The relative amount of DNA in arbitrary units was calculated as in the testis by (E_m/F).

RESULTS AND DISCUSSION

The haploid spermatid nuclei that were measured were nearly all spherical, very homogenous in density, and gave very uniform readings (table 1 and 2), thereby providing an excellent standard. The relative amount of DNA in a nucleus is quantified by some C-amount of DNA which it contains. As the spermatid has 1N set of chromosomes, it also contains 1C amount of DNA. The diploid secondary spermatocytes, used as a check, gave calculations very close to the expected multiple over the standard, as they contain the 2C amount of DNA. Good readings were obtained from both species of cockroaches (table 2).

TABLE 1

Nuclear size measurements from various tissues of the American and German cockroaches

Material examined	<i>Periplaneta americana</i> (L.)		<i>Blattella germanica</i> (L.)	
	Number of nuclei	Mean nuclear diameter (μ) and S.D.	Number of nuclei	Mean nuclear diameter (μ) and S.D.
Testis				
Spermatids	41	2.68 \pm .06	6	2.61 \pm .02
Secondary spermatocytes	5	2.94 \pm .13	10	2.80 \pm .07
Accessory gland				
Small nuclei	5	2.81 \pm .07 \times 11.29 \pm .24 ¹	11	2.66 \pm .06 \times 7.59 \pm .23
Large nuclei	8	2.85 \pm .09 \times 16.11 \pm .32	6	2.72 \pm .08 \times 10.89 \pm .21

¹Short and long diameters respectively for all accessory gland nuclei.

Calculations on the nuclei of unknown ploidy, if reliable, should be reasonably near some multiple of the spermatid C-value, corresponding to their appropriate N-number. Although unusual multiples of the chromosome number occur in nature (i.e., 3N, 5N, polysomics, etc.), the normal multiple follows the geometric progression (i.e., 2N, 4N, 8N, 16N, etc.). Examination of the accessory gland calculations in table 2 shows that as multiples of the standard they are very close to the numbers in the geometric series. As expected from the two diameter classes observed, two distinct classes of ploidy were found in each species of cockroach. Apparently, *P. americana* contains 16-ploid and 32-ploid nuclei and *B. germanica* contains 8-ploid and 16-ploid nuclei. Perhaps other classes were present, but they were not observed.

According to White (1954), endopolyploidy seems to be a characteristic of all insects that have been studied cytologically, and is probably an essential element in their mechanism of histological differentiation. During development, insect volume increases many times, but mitosis occurs only rarely in some tissues, and not at all in others. Thus there is a great enlargement of the individual cells and their nuclei while there is only a slight increase in cell number.

Although studies on accessory gland development were not made, the large

nuclei probably arose through endomitosis. No chromosomes were observed in the nuclei, but as the insects were mature or nearly so, accessory gland development could have been completed also. More definitive studies certainly should bear this out.

Some researchers have reported various degrees of positive correlation between the secretory activity of a tissue and the degree of ploidy (Merriam and Ris,

TABLE 2
Cytophotometric measurement data and the estimated ploidy of various nuclei in tissues of the American and German cockroaches

Material examined	Number of nuclei measured	Mean relative amount of DNA	Multiple of standard ¹	Estimated N number
<i>Periplaneta americana</i> (L.)				
Testis				
Spermatids	41	.099 ± .002 ²	1.00	1
Secondary spermatocytes	5	.200 ± .010	2.02	2
Accessory gland				
Small nuclei	5	1.626 ³	16.42	16
Large nuclei	8	3.135	31.67	32
<i>Blattella germanica</i> (L.)				
Testis				
Spermatids	6	.081 ± .002	1.00	1
Secondary spermatocytes	10	.164 ± .002	2.02	2
Accessory gland				
Small nuclei	11	.681	8.39	8
Large nuclei	6	1.386	17.11	16

¹Standard is the haploid spermatid.

²Standard error.

³All accessory gland readings from pooled data.

1954; White, 1954). Swift and Rasch (1956) reported a geometric series of 10 DNA classes ranging from diploid to 1024-ploid in the nuclei of the salivary glands of *Drosophila*. Because of the large size of the cockroach accessory gland and the spacious lumens in its utriculi, one might suspect that this gland is also highly secretory. The high degree of ploidy of the accessory gland nuclei, therefore, may be due to its cell function.

Non-spherical nuclei are seldom chosen in normal photometric work, since various errors may be introduced in the intensity readings and calculated core volume. Known techniques for measuring irregular nuclei are the scanning method (Casperson 1936) and the two-wavelength method (Patau, 1952), but no simple one-wavelength methods are known for oblate spheroids. Since the data on the accessory gland nuclei obtained in this study gave apparently reliable DNA calculations, one might reasonably assume that the modified technique developed here is a valid method of estimating the degree of ploidy—at least in the normal geometric-series range. Determinations of aneuploids are beyond the scope of this technique.

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